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Hibernation, stress, intestinal functions, and catecholamine turnover rate
in hamsters and gerbils.

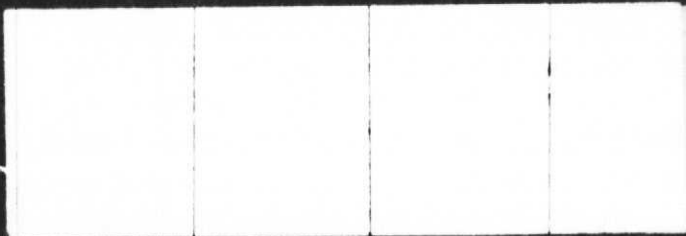
(NASA-CR-146662) [HIBERNATION, STRESS,
INTESTINAL FUNCTIONS, AND CATECHOLAMINE
TURNOVER RATE IN HAMSTERS AND GERBILS]

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ANNUAL STATUS REPORT AND
REQUEST FOR CONTINUATION

NASA Grant NGL 26-004-C21

X. J. Musacchia, Ph.D.

(August 1973)

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INTRODUCTION

In compliance with the Research and Technology Operating Plan (RTOP) issued May 30, 1973, proposed research projects in five (5) areas are herein described. In these projects we propose to bridge the ongoing research in depressed metabolism with gravitational biology. The "new" orientation is not difficult since gravitational biology is in reality a facet of environmental biology and our research interests have in fact been environmental physiology. Our efforts will be concentrated on projects which are encompassed by the three primary areas: (1) bioenergetics (metabolism); (2) thermoregulation; and (3) cardiovascular parameters.

In designing these projects we asked the questions: (1) What are the most significant extraterrestrial environmental variables which can effect physiological functions; (2) Can animal models or experimental design be devised to test specific effects; and (3) What are the definable limits (stress, adaptation, etc.) to which mammalian systems can be subjected.

The experimental design in each project is focused on assessing altered metabolic and functional responses when an animal is exposed to hypergravity, normogravity and hypogravity. Comparison of effects of increased gravitational forces and normal gravity can be assessed using earth side laboratory facilities. The zero-G effects will require extraterrestrial vehicles. In addition, each project is designed to make the best use of ongoing projects in which problems of thermoregulation, hemodynamics, gastrointestinal function and neuroendocrine meta-

bolism due to altered metabolic states (e.g., hypothermia, heat stress, etc.) are intimately associated with ongoing programs in gravitational biology.

In brief, the transition from an emphasis in depressed metabolism to altered metabolism due to varied gravitational forces is readily achieved. There are many features of the experimental systems (e.g., micro cannulations, small mammal metabolism, cardiovascular measures and hemodynamic systems, development of non-invasive telemetry systems) devised for hamsters in depressed metabolic states which lend themselves to protocols designed to answer questions concerning gravitational biology.

1. RENAL FUNCTION AND RESPONSE TO STRESSFUL ENVIRONMENTS

In these series of experiments the environmental stress will be represented in two ways: depressed metabolism due to hypothermia and hypergravitational forces due to centrifugation. A range of effects will be measured using kidney preparations from hamsters.

The initial project involves an assessment of the functional state of the kidney through determinations of cortico-medullary concentration gradients of: sodium, potassium and urea. The objective of these experiments is to determine whether or not filtration is occurring or is altered due to physiological insult. Initially, the type of stress will be hypothermia; a second family of experiments will involve hypergravitational forces and a third form of experimental approach will involve heat stress. Ultimately, the objectives will include animals maintained at zero G for varying periods.

It is reasoned that the presence of a gradient for any or all of the above components implies the presence of glomerular filtration, whereas absence of a gradient suggests a reduction or elimination of glomerular filtration. The kidney-slice technique which will be used, permits one to assess whether filtration is or is not occurring, although it does not provide quantitation. Attempts to quantitate filtration will be done with classic clearance techniques. Perfection of clearance techniques with small mammal preparations is being worked on in several laboratories. Our personnel are already adept at miniaturization and cannulation procedures in blood vessels in hamsters and rats; we foresee the only obstacles to be modification of large mammal methods to small mammal models.

Cortico-medullary studies are being done in: (1) control hamsters $T_{re} = 37^{\circ}\text{C}$; (2) hypothermic hamster $T_{re} = 7^{\circ}\text{C}$; (3) rewarming hamster $T_{re} = 18^{\circ}\text{C}$; and (4) rewarming hamster $T_{re} = 37^{\circ}\text{C}$ for about 2 hours following 48 hours at $T_{re} = 7^{\circ}\text{C}$. Methods for determinations of effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) under conditions of normothermia and hypothermia are currently being developed.

Upon successful development of these methods and the achievement of data collection processes, we will attempt to collaborate in an experiment utilizing hyper G forces. The centrifuge requirements will be discussed and reviewed with Dr. Jiro Oyama and other participants in the consortium team.

Resumé: The studies outlined in the above section are intended to continue implementation of the RTOP Operating Plan, section 5 and 10. and they are also aimed at providing a means to study (2) "basic course of electrolyte imbalance" and (8) "the adverse reactions to metabolic extremes" and how they can be modified or eliminated".

2. ROLE OF SYMPATHETIC CAPACITY IN THERMOREGULATION WITH RELATION TO ALTERED ENVIRONMENTS

Modification of the sympatho-adrenal system is an ideal approach to understanding neuroendocrine mechanisms involved in adaptations to various stresses and, in particular, various G levels.

One of our current projects is aimed at determining the role of sympathetic capacity in thermoregulation at different states of temperature acclimation. Recent advances in the development and characterization of chemical agents which cause permanent or temporary sympathectomy provide a convenient tool for this purpose. Initial studies of chemical sympathectomy using 6-hydroxy dopamine, require the derivation of adequate dose response curves for the hamster. This study, presently in progress, will be followed by measurements of specific parameters of body temperatures, O_2 consumption, blood pressure, hematocrit and capacity to withstand further elevations or depressions of ambient temperature in assessing sympatho-adrenal activity.

Whereas our initial studies will involve temperature stress as the environmental variable, forthcoming interest will be concerned with increased G forces and, in addition, we will have improved the animal model for investigation of zero G effects.

We reasoned that this work is highly significant since insight into the importance of sympathetic activity in temperature acclimation up to this time has been limited chiefly to surgical sympathectomy and blockade of specific adrenergic receptors. The employment of chemical sympathectomizing agents in this environmental situation

has not previously been reported. In addition, this technique has not been applied to the hamster nor to other small laboratory mammals.

Another feature about our experimental approach is that it provides a non-invasive method for studies of short term and long term stressful situations. Ultimately the animal is sacrificed for analytical purposes; however, administration of the drug in varying dosages and for varying periods should assist the consortium group in considerations of space flight objectives.

Resumé: These studies are intended to assess the role of neuroendocrine factors in response to environmental variables: viz. temperature and gravity. The animal model under initial testing is the hamster and the environmental variable is temperature. These experiments are intended to facilitate the RTOP for Regulatory Biology and my T-4; in particular sections (2) "The central nervous system; its control of thermoregulation", and (3) "The endocrine system; its role in adaptation to various G levels".

3.

CATECHOLAMINE TURNOVER IN RESPONSE TO ENVIRONMENTAL STRESSES

In an initial study, non-isotopic and isotopic determinations of catecholamine turnover rates of the golden hamster will be made under varying conditions of ambient temperatures.

The experimental approach will include inhibition of synthesis of endogenous catecholamines with α -methyl-p-tyrosine, which allows the rate of utilization to be determined by measuring the decay of tissue levels of catecholamines over a period of hours. In conjunction, the labelling of endogenous tissue catecholamine stores with isotopic compounds (H^3 -Dopa) and their disappearance with time provides a quantitative measure of turnover rates. Heart, kidney and adrenal tissue will be studied in hamsters at acclimation temperatures T_a 22°C and T_a 34°C. The former represents room temperature and the latter an experimental high temperature.

The rationale for these experiments is based on several factors which are important to the NASA Regulatory Biology Program. Neuroendocrine responses to environmental stresses are recognized as essential to our understanding of mammalian responses to a gravity free state, to increased G forces and to severely altered environmental temperatures.

The role of catecholamine synthesis and release in cold acclimation has been widely studied in rodents. The physiologic importance of catecholamines in both normothermic and cold-acclimated animals is recognized as an integral component of homeostasis. Bioamines, specifically norepinephrine (NE) and epinephrine (E) are also essential in mammalian re-

sponses to stress of either short or long duration. High temperature acclimation in rodents would also appear to rely on some specialized physiologic capacity. The importance of catecholamines in this situation, has not been described. Acclimation to zero G and/or to increased G forces have not been well investigated in terms of the role of catecholamines.

It is our objective to investigate first the heat acclimation and heat stress problems, and then to initiate a project using animals exposed to increased G forces. Centrifugal effects will necessitate closer alliance with those members of the NASA consortium who have centrifuge facilities. This prospect should be explored during the next meeting of the NASA Regulatory Biology Group.

In addition, a limited project concerned with brain catecholamines has been initiated and will be expanded along the experimental lines described for heart, kidney and adrenal.

Resumé: These studies will be used as an approach to investigate distinct endocrine systems, in particular, catecholamines. Comparisons and similarities of responses due to several environmental variables will be evaluated. The environmental variables include: cold (which includes a substantial literature) and will not be repeated to any extent in our studies; heat which has been relatively unexplored and will be a major thrust in our studies; and increased G forces which will include collaboration with other members of the consortium.

Changes in hemodynamics in mammals subjected to conditions of stress resulting from exposure to a harsh environment or in the processes of acclimation are readily predictable. Little is known about changes in circulation, blood pooling, hemostasis and other hemodynamic features in animals subjected to increased G forces, hypothermia and, for that matter, other forms of altered and possibly stressful environments.

We propose to study the distribution of blood elements in hypothermic hamsters. Using an animal system with reduced cardiovascular responses should provide a means of investigation under conditions of greatly slowed functional capacity. Comparisons would be made with normothermic subjects and plans initiated for investigation of animals exposed to varying centrifugal forces.

These studies will be done by labelling red cells with ^{99m}Tc and/or labelled albumin. It is anticipated that red cell distribution can be followed by external monitoring. Remote sensing in these animals will be performed using a Nuclear of Chicago Scintillation Camera located in the Radiology Department of the Medical School, UMC. The initial experiments would include tracing the red cell distribution as the animal rewarms from hypothermia. Results from those studies will provide information about relative perfusion of tissues (with red cells) at various body temperatures.

Two steps have already been accomplished: (1) we are able to transfer red blood cells in hamsters via jugular cannulas and (2) the method of Eckelman et al. for labelling red cells with ^{99m}Tc has been

accomplished. In the Fall of 1973 these procedures will be developed so that labelling of small volumes of cells will be achieved.

We are of the opinion that if these methods can be developed with one type of animal, they may well become highly utilitarian in studies with a wide variety of animals subjected to various types of environmental stresses. The methods are to some degree non-invasive and will permit long term use of animals.

Resumé: This project provides an approach to problems in hemodynamic alterations due to exposure to stressful environments. A blood labelling system will be employed. These types of studies provide substantial approaches to goals listed in the Operating Plan (1) "The cardiovascular system; its responses to weightlessness and hypergravity" and to other pertinent questions relevant to cardiovascular and hemodynamic changes.

This study is part of a broad investigation by this laboratory of the effects of a variety of environmental stresses (heat, cold, altitude and exposure to ionizing radiation) on intestinal function.

In comparison to the normothermic animal, heat stress causes a decrease in food intake and an alteration in calorogenesis which appears to be mediated by hormonal control. The effect of heat stress on the absorption of nutrients has not been well studied although there are several incomplete reports which suggest it may be altered. Changes in circulating hormone levels and dietary restriction may alter glucose absorption (Wiseman, 1964). Pituitary, adrenal and thyroid hormone levels may alter growth rates of intestinal tissue and hence absorptive capacity. Finally, changes in electrolyte levels associated with heat stress, increased G forces and other forms of environmental stress may alter Na dependent active transport of glucose and other materials.

The purpose of these investigations is to determine the extent of alteration of active transport of glucose in the hamster by both acute and chronic exposure to ambient temperatures circa 33°C and to determine if these effects are dependent on changes in turnover rates of intestinal cells. The following preliminary study is in progress. Groups of hamsters are exposed to 33°C for periods of 2 days to 6 weeks. Active transport of glucose is measured by the technique of Crane and Mandelstam (1960). This consists of determining uptake of sugars in in vitro tissue slices. Two series of experiments are planned, uptake

of glucose and uptake of the non-metabolizable sugar, 3-O methyl glucose. Turnover rate will be measured by autoradiography as described by Meissier and Leblond (1960). It is anticipated that the same experimental protocol will be used in experiments concerned with the effect of increased G forces. The present system will provide an animal model and technical approach to problems in gravitational biology.

Resumé: Intestinal function, in terms of glucose absorption, will be examined in animals subjected to a stressful environment, e.g., heat and/or hypergravity (centrifugation). These projects attempt to explore the area cited the Operating Plan, namely (4) "The gastrointestinal system as influenced by G". In addition, it is argued that assessments of other systems are limited until a clearer understanding of the animal's gastrointestinal capacity to absorb nutrient matter is obtained.

PROPOSED BUDGET

Supplement 10 NASA NGL 26-004-021

Salaries

Research Associate @\$10,000/annum 11/1/73 through 9/30/74 - 100% FTE	\$ 9,167
Technician - 100% FTE	8,000
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SUBTOTAL S&W	\$17,167
Fringe Benefits (14% S&W)	2,403
Indirect Costs (58.17% S&W)	9,986
	<hr/>
SUBTOTAL S&W plus INDIRECT COSTS	\$29,556
Less Cost Sharing by University	- 591
	<hr/>
	\$28,965

II. Progress Reports of Recent Achievements

A brief summary of research projects during the last fiscal period of NASA Grant NGL 26-004-021, starting July 1, 1972 to the present, is provided herein. This research has been carried out in the Dalton Research Center, University of Missouri - Columbia. Use of the Center's facilities are of inestimable value in achieving success in this research.

The results of experiments done in collaboration with Dr. Cecil Entenman, Berkeley, California, have been described in reports from the Institute for Lipid Research.

The request for continuation funds during the coming year is summarized in the budget sheet (page 13). Remaining funds (approximately \$8,000 in September 1973) and commitment to purchase orders will be used in accordance with original designations as provided in active Supplements to NGL 26-004-021. In effect, these funds will be used in a continuation of grant commitments: to purchase expendable items (e.g., reagents, glassware, radioactive materials, etc.) and to defray expenses involved in publications, repairs to equipment, travel to national and scientific meetings, and to consult with other members of the consortium. No requests for items such as those cited above will be made in the budget for the 1973-1974 supplement.

The main thrusts during the next year will be:

- (1) an emphasis on modifying our experimental approaches to studies in gravitational biology;
- (2) a search for similarities in physiologic responses under a variety of environmental states (e.g., increased G forces, heat, cold, etc.);

- (3) additional collaboration and interaction with other members of the consortium.

1. Bioenergetics of Altered Metabolic States (depressed metabolism)

The objective was to extend survival time in the helium-cold hypothermic hamster. By means of external support of blood glucose as an energy source, the initial objective has been achieved. The glucose supported animals survive at T_{re} 7°C for periods of three and four days. This represents a three to four fold increase in survival as compared with the non-supported animal.

Utilization of glucose under depressed metabolic conditions was initiated using chronically cannulated animals in which blood glucose levels were monitored. Comparisons with animals infused with a non-metabolizable sugar (3-O-methyl glucose) are currently being made.

A second feature of this research is aimed at clarifying and defining "clinical survival" and "biological survival".

This research has been done in collaboration with Mr. Garth Resch, a doctoral student in the Department of Physiology.

Two papers have been presented at (1) the FASEB, a national meeting of the American Physiological Society (April, 1973) and before (2) the Missouri Academy of Sciences (April, 1973).

2. Development of Telemetry Transmitting System

The technicians in the Electronics Shop in the Dalton Research Center, University of Missouri - Columbia, have been working on a telemetry system to transmit information concerning body temperature and EKG. The circuitry being used is from a NASA publication and our modifications are aimed at miniaturization.

Currently the hamster is being used as the experimental animal. It is anticipated that the effects of stressful environments (varied G forces, heat and cold) will be assessed in terms of perturbation in body temperature regulation and cardiovascular alteration. Small, miniaturized telemeters can provide a long term means of gathering information about functional systems via non-invasive techniques.

We consider the development of telemetry systems for small mammals essential to all members of the NASA Regulatory Biology Consortium.

Dr. George Tempel is collaborating in this project.

3. Comparative Radioresistance of Hibernating and Hypothermic Hamsters

The objective was to make a comparison of the levels of radioresistance achieved with two states of depressed metabolism: hibernation and hypothermia. Our research and the publications in this area have clearly established that with hibernation and hypothermia, animals subjected to whole body ionizing radiation show dose reduction factors of 1.2 to 1.4.

We are in the terminal stages of a project concerned with dose response curves (700 rads to 3000 rads) using the hibernating hamster. These experiments will be unique in that a direct comparison between a hibernating and hypothermic animal of the same species has never been performed.

The long term project concerned with synergistic effects of temperature and lethal and sub-lethal doses of ionizing radiation will be continued.

Dr. Wynn Volkert has collaborated in these projects.

4. The Effect of Environmental Stress on Intestinal Transport in the Hamster

This study has been based on three questions concerned with the response of the intestine to environmental variables. The intestine is viewed as a primary factor in the maintenance of physiological homeostasis:

- (1) Does acute or chronic exposures of an animal to altered environments cause changes in the capacity of the intestine for active transport of glucose?
- (2) If an alteration in function does occur, what is its extent and time course? (Are "early" changes due to stress and "late" changes due to acclimation?)
- (3) Is the mechanism of change in function related to developmental alterations and changes in rate of turnover of cells in the intestinal mucosa?

A pilot study has been underway using hamsters exposed to heat stress (T_a 34°C) for one day to six weeks. In vitro preparations are being used to determine levels of glucose active transport.

This project is in progress and is being developed by Mecca Carpenter, a doctoral student in the Department of Physiology.

5. Radioresistance and Intestinal Function in the Gerbil

The objective of this project was to relate the high levels of radioresistance in the gerbil, Meriones unguiculatus, to a functional parameter. We selected gastrointestinal function and, specifically, absorption of glucose.

Our results clearly showed that the LD_{50/30} of 1250 rads is much higher than that in the more common laboratory species (rats, mice, etc.) and compares favorably to that in ground squirrels and hamsters.

Again, in contrast to rats and other mammals, in vivo intestinal absorption in gerbils one and two days following whole body exposures to lethal levels of radiation is not significantly modified. In fact, post irradiation absorption at periods of one or two weeks is not greatly altered. Measurements were made using both in vivo and in vitro systems. The data suggest that the increased levels of radio protection were due in part to the ability of the gut to maintain functional integrity.

Whether this increase in radio protection can be assessed in terms of the mitotic capacity or a peculiarity of the absorptive mechanism is not yet answered. We consider that we have at least provided a first step, in terms of functional attributes, in exploring the nature of radioresistance in the gerbil.

6. Determination of Catecholamine Turnover Rates in the Hamster

Acclimation to high temperature in rodents appears to rely on specialized physiologic capacities and our interests have focused on the role of the bioamines.

The role of catecholamine synthesis and release in cold acclimation has been widely studied. The physiologic importance of catecholamines in homeostasis is well recognized. Bioamines, specifically norepinephrine (NE) and epinephrine (E) are known to be essential in mammalian responses to stress of either short term or long term duration.

During the past year we have employed a method of measurement of catecholamine turnover rate using α -methyl-p-tyrosine. The procedure provided experimental data, for the hamster, which are comparable to that reported in the literature for the white rat.

A selected experiment was done using hamster ventricle tissue. The results showed marked changes in animals acclimated to 34°C. The comparisons were made with control animals maintained at 22°C. The data are being analyzed.

We are confident that the model system employed in heat stress studies will serve in studies of other forms of experimental stress, e.g., increased G forces.

A physiology doctoral student, Mr. Steve Jones, has been involved in developing this project. He presented a paper before the American Physiological Society in August, 1973.

III. Appendix

Articles and Publications

Helium-cold induced hypothermia in the white rat. X. J. Musacchia and Mollie Jacobs. Proc. Soc. Expr. Biol. Med. 142:734-739, 1973.

Hypoxia, an adjunct in helium-cold hypothermia: sparing effect on hepatic and cardiac metabolites. G. L. Anderson, G. E. Resch and X. J. Musacchia. Am. J. Physiol. 224(1):144-147, 1973.

Mechlorethamine effects on intestinal absorption (in vitro) and cell proliferation. M. A. Wurth and X. J. Musacchia. Am. J. Physiol. 225:73-80, 1973.

Abstracts of Papers Presented

Metabolic characteristics in hamsters during long term helium-cold hypothermia and recovery. G. E. Resch, X. J. Musacchia, and W. Volkert. Federation Proceedings 32(3):375 Abs., 1973.

Effects of argon on mammalian metabolism. G. E. Tempel and X. J. Musacchia. Bull. Mo. Acad. Sci. 1(5):63, 1973.

Effects of temperature acclimation on endogenous tissue levels of catecholamines in the hamster. S. B. Jones and X. J. Musacchia. Bull. Mo. Acad. Sci. 1(5):63, 1973.

Thyroid calcitonin levels in the hibernating ground squirrel. X. J. Musacchia and A. D. Kenny. The Endocrine Society: Program of the 55th Annual Meeting, June 20-22, 1973, Conrad Hilton, Chicago, Illinois, 1973.

Renal function in helium-cold hypothermic hamsters (M. auratus). G. E. Tempel and X. J. Musacchia. The Physiologist 16(3):468, 1973.

Effects of temperature acclimation on catecholamine tissue levels and sympathetic capacity in the hamster. S. B. Jones and X. J. Musacchia. The Physiologist 16(3):355, 1973.